PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Shogo ISHIUCHI

Appln. No.: 10/509,379

Confirmation No.: 5052

Filed: September 27, 2004

For: REMEDY FOR GLIOBLASTOMA

Docket No: Q83588

Group Art Unit: 1612

Examiner: Snigdha MAEWALL

SUBMISSION OF EXECUTED DECLARATIONS UNDER 37 C.F.R. §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith is a copy of (1) a re-executed Declaration Under 37 C.F.R. §1.132 signed by Shogo ISHIUCHI on September 3, 2009 (An original Declaration was executed March 12, 2009, but contained typographical errors; the currently submitted Declaration corrects those typographical errors); and (2) a second Declaration Under 37 C.1.132 signed by Shogo ISHIUCHI on September 3, 2009. Consideration and entry of the Declarations are respectfully requested.

Respectfully submitted,

/Sunhee Lee/

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Date: October 1, 2009

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For: REMEDY FOR GLIOBLASTOMA

SECOND DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Shogo ISHIUCHI, hereby declare and state:

THAT I am a citizen of JAPAN;

THAT I am the same Shogo ISHIUCHI who executed the Declaration under 37 C.F.R. § 1.132 on March 12, 2009, which was submitted on March 20, 2009, in this application;

THAT I have received the degree of M.D. in 1985[1] from Gunma University;

THAT I have been employed by University of the Ryukyus since June 1, 2009, where I hold a position as professor of Department of Neurosurgery, Faculty of Medicine, with responsibility as Chairman; and was employed by Department of Neurosurgery, Gunma

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[1] I noticed a typographical error in the Declaration executed March 12, 2009: the term "1998" (i.e., the year when I received the degree of M.D.) should read "1985."

University School of Medicine from 1999 to 2009, where I held the position as Associate Professor with responsibility for Medical Director.

I wish to correct some minor typographical errors in the Rule 1.132 Declaration that I executed on March 12, 2009 and was filed on March 20, 2009. The Declaration should be corrected to state that I have received the degree of M.D. in 1985, not 1998, from Gunma University. Also, the spelling of "Nuerosurgery" in line 5 should be corrected. In order to correct the typographical errors, I submit herewith a corrected version of the March 20, 2009 Rule 1.132 Declaration, which is re-executed.

I declare that I am familiar with the Office Action mailed June 9, 2009. On page 3 of the Action, claims 13 and 18 are rejected under 35 U.S.C. 112, first paragraph, because allegedly the specification, while being enabling for *in vitro* treatment of glioblastoma, does not reasonably provide enablement for *in vivo* treatment of glioblastoma with each and every compound claimed. On page 9 of the Action, claims 13 and 18 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Andrasi et al. (USP 5,639,751) in view of Takano et al., ("Glutamate release promotes growth of malignant gliomas", Nature Medicine, 7 (9), pp. 1010-1015 (2001) presented in IDS) and Catarina L. Florian et al., ("Characteristic metabolic profiles revealed by IH NMR spectroscopy for three types of brain and nervous system tumours", NMR in Biomedicine,Vol. 8, pp. 253-264 (1996) presented in IDS).

In order to show that the correlation of *in vivo* and *in vitro* test results of the claimed compounds (in particular, those fall into the scope of claim 13, and those recited in claims 13-18), Tests 2 and 3 described in the following pages were performed by me or under my supervision. Test 1 was conducted in order to determine the activity of MK801, which is

reported in Takano reference (one of references cited by the Examiner in rejecting claims of the instant application under 35 U.S.C. § 103).

The compounds employed in the Tests are as follows:

MK801 (Test 1): An NMDA antagonist

Compound B (Test 2): 2-[N-(4-chlorophenyl)-N-methylamino]-4H-pyrido[3,2-e]-1,3-thiazin-4-one (claim 16 of the application)

GYKI52466 (Test 3): 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (claim 17 of the application)

The experimental method used in Test 1 is the same as that used in the previously filed Rule 132 Declaration (submitted March 20, 2009), the experimental method used in Test 2 is the same as that used in Example 2 in the specification of the instant application; and the xenograft model of Test 3 is the same as the model mentioned in Example 3 in the specification of the instant application.

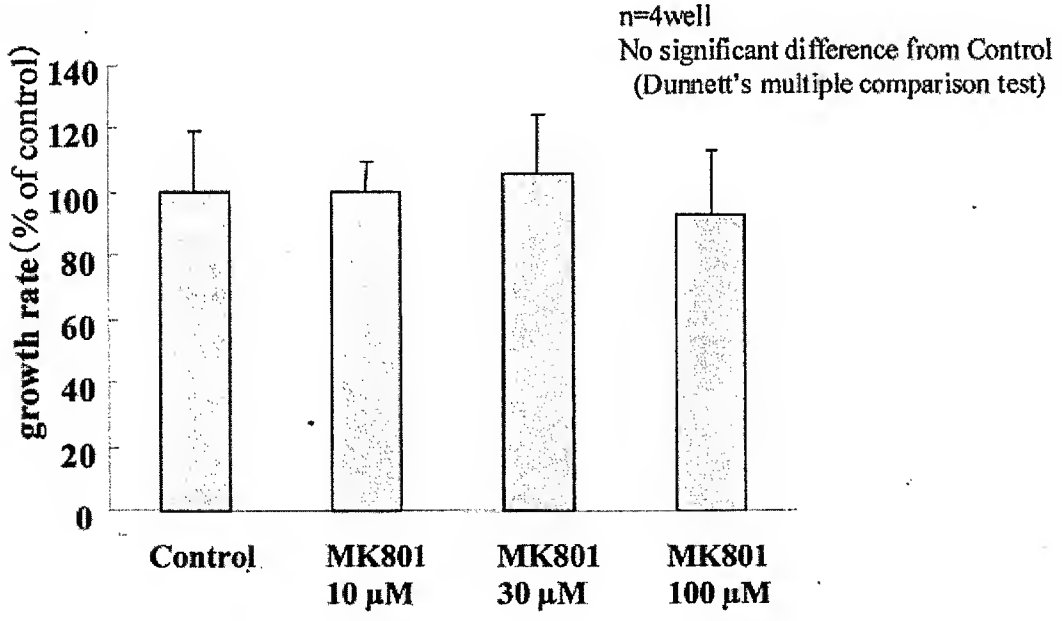
Test 1 Inhibitory effects of NMDA antagonist MK801 on glioblastoma growth
-in vitro-

Human glioblastoma cell (CGNH-89 cell line) was used at this experiment. The cells were inoculated at 1 x 10^5 cells per well into well plates containing Eagle's medium with 5% serum. These cells were randomly divided in the following four groups 1 day after seeding: control group, 10μM MK801 group, 30μM MK801 group, and 100μM MK801 group. The cells were incubated for 96 hours. The CGNH-89 cell was cultured in DMEM (Dulbecco's modified Eagle's medium) with glutamine-free and glutamate-free. Each group was set at 4 wells.

The anti-tumor action was evaluated by determining cell count per well using a hemocytometer 96 hours after culturing. The results are shown as mean ± standard error and statistically analyzed by the Dunnett's multiple comparison test.

As shown in **Figure 1**, NMDA antagonist MK801 showed **no** significant inhibitory activity against the growth of CGNH-89 cells **even at 100μM**.

Figure 1



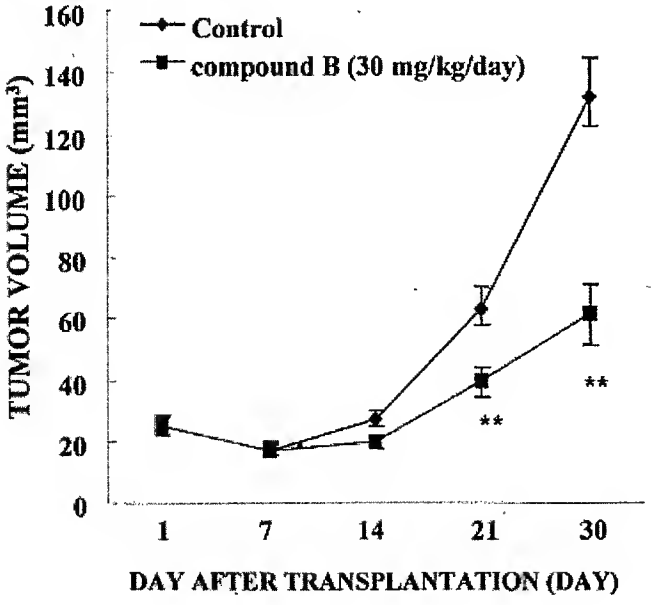
Test 2 Inhibitory effects of compound B on glioblastoma growth *-in vivo-*

Nude mice (5 weeks old) were used at this experiment. 5×10^6 cells of CGNH-89 were transplanted subcutaneously in the nude mice. On the fourth day of transplantation, the mice were divided randomly in two groups; a group (n=9-10) to be administered with 30 mg/kg compound B and a group (n=9-10) to be administered with PBS (phosphate buffered saline). The drug was administered repeatedly orally for 28 days starting on the fourth day of transplantation. The tumor size was measured every 7 days with a vernier micrometer to calculate the volume according to the formula (length x width^2) x $^1/_2$.

The results are shown in mean ± standard deviation and statistically analyzed by the Student's t-test. The significance level was set at $p<0.05$.

Compound B as an antagonist against AMPA receptor showed a significant tumor growth inhibition on days 21 and 30 after the onset of administration **(Figure 2)**

Figure 2



n=9-10
**p<0.01
significant difference from Control
(Students't test)

Test 3 *In vivo* effect of GYKI 52466 in xenograft model

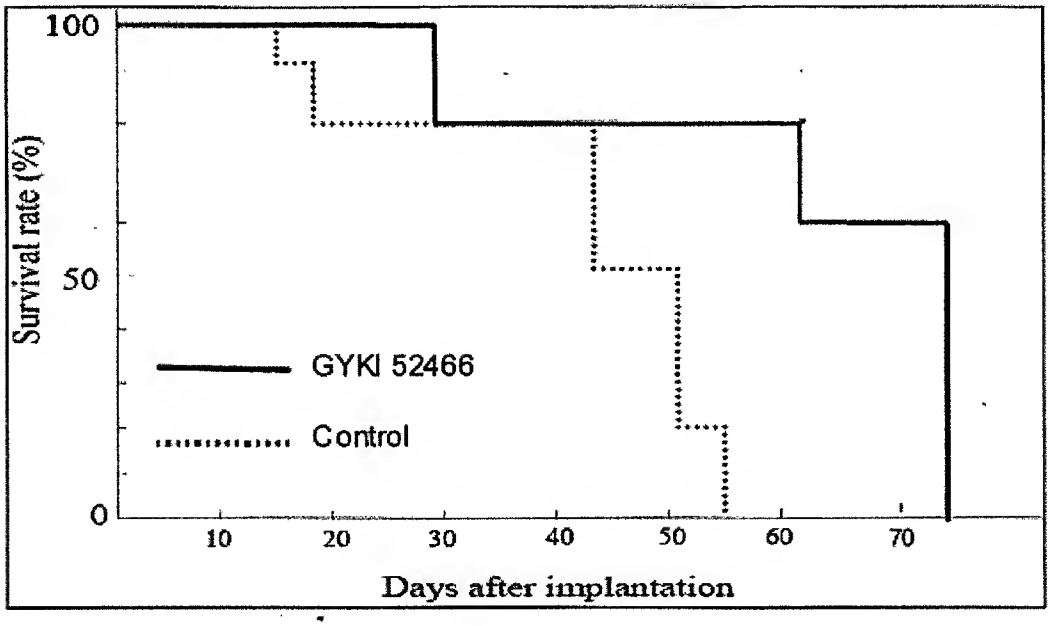
(Method)

Xenograft experiments.

Intracranial implantation of CGNH-89 cells were performed as previously described (Ishiuchi et al., Nature Medicine, 8(9), 971-978 (2002)).

As shown in **Figure 3**, in xenografted nude mice model, once-daily usage of GYKI 52466 (25 mg/kg, intraperitoneal administration) during 60 days after implantation prolonged survival term (63.0 ± 5.7 days, n=10) compared to non-treated control (41.8 ± 4.6 days, n=10) ($p<0.01$, Bonferroni/Dunn).

Figure 3



I also wish to incorporate by reference in its entirety the *in vitro* experimental results of Compound A included in a 2002 publication [Ishiuchi et al., "Blockage of Ca^{2+}-permeable AMPA receptors suppresses migration and induces apoptosis in human glioblastoma cells" Nature Medicine, 8(9), 971-978 (2002)], of which I am the primary author. A copy of the 2002 publication is submitted under a separate transmittal letter.

Specifically, on page 974 of the 2002 publication, in the right column, *in vitro* experimental results of Compound A are shown. The experimental method used to obtain these results is the same as that used in Example 1 in the specification of the present application.

Based on the experimental results shown in the instant Declaration, the previously submitted Declaration (executed March 12, 2009 and submitted March 20, 2009), and the disclosure of the specification of the above-identified application, I respectfully submit that the correlation between *in vitro* and *in vivo* effectiveness of representative examples of the compounds recited in claim 13 as well as those recited in claims 14-18 are established.

In the meantime, the result of Test 1 employing MK801 of Takano reference demonstrates that MK801 fails to inhibit proliferation of human glioblastoma cells.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: September 3, 2009

Shogo Ishiguchi